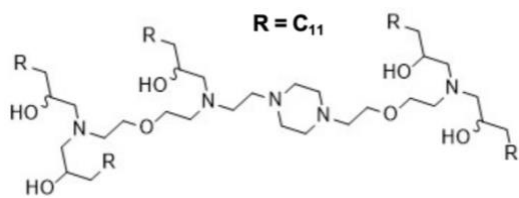
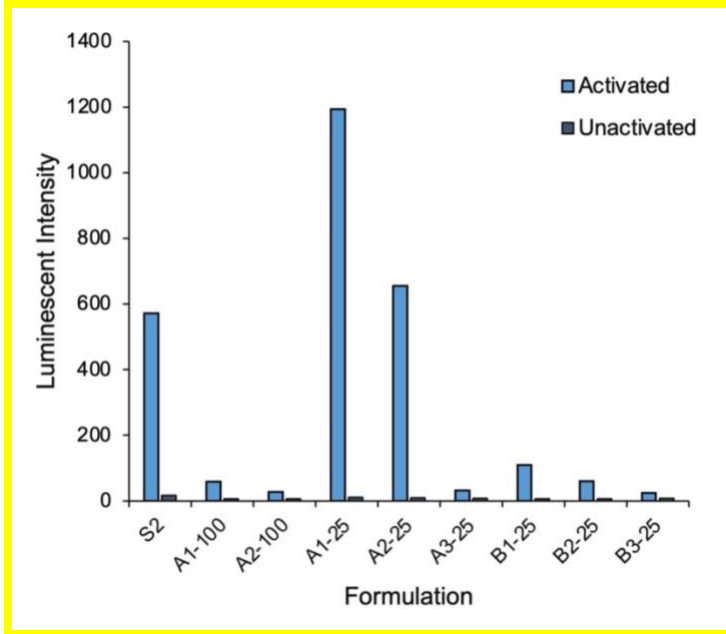


LNP	Encapsulation Efficiency (%)	Z-Average Size (nm)	PDI	Zeta Potential (mV)	pKa
S2	91	90.6 ± 29.31	0.19 ± 0.07	-4.36 ± 3.73	5.9
A1-12.5	96	59.35 ± 7.43	0.2 ± 0.03	-6.9 ± 0.8	5.92
A2-12.5	96	84.88 ± 4.61	0.08 ± 0.07	-17.33 ± 2.46	5.79
A3-12.5	96	82.05 ± 2.34	0.17 ± 0.04	-5.75 ± 2.88	6.02
B1-12.5	95	61.03 ± 1.18	0.26 ± 0.02	-16.57 ± 2.48	5.97
B2-12.5	95	86.95 ± 3.91	0.07 ± 0.05	-8.48 ± 1.67	5.79
B3-12.5	95	77.52 ± 5.98	0.28 ± 0.02	-3.84 ± 1.21	6.17
A1-25	90	84.44 ± 16.88	0.19 ± 0.09	-8.82 ± 3.86	6.19
A2-25	91	78.52 ± 18.28	0.25 ± 0.04	-16.89 ± 10.8	6.03
A3-25	94	68.52 ± 1.79	0.22 ± 0.04	-10.75 ± 5.35	6.12
B1-25	95	95.1 ± 15.12	0.23 ± 0.03	-9.33 ± 1.07	5.91
B2-25	95	62.44 ± 2.66	0.25 ± 0.05	-10.18 ± 1.24	6.05
B3-25	94	75.39 ± 2.09	0.29 ± 0.03	-7.07 ± 0.25	6.08
A1-50	95	102.15 ± 16.97	0.23 ± 0.08	-2.36 ± 0.43	6.48
A2-50	95	115.68 ± 24.44	0.17 ± 0.12	-4.2 ± 0.36	6.16
A3-50	95	103.12 ± 7.73	0.18 ± 0.01	-9 ± 0.63	6.43
B1-50	96	97.6 ± 22.94	0.21 ± 0.03	-16.23 ± 1.08	6.12
B2-50	95	100.07 ± 6.55	0.36 ± 0.09	-9.68 ± 1.55	6.7
B3-50	95	138.7 ± 35.24	0.31 ± 0.07	-4.83 ± 0.88	6.2
A1-100	92	88.08 ± 10.05	0.22 ± 0.06	1.67 ± 1.13	6.44
A2-100	88	89.45 ± 13.88	0.19 ± 0.05	-2.46 ± 0.27	6.25
A3-100	91	81.79 ± 9.16	0.19 ± 0.07	-3.58 ± 3.7	5.73
B1-100	92	85.72 ± 6.89	0.17 ± 0.06	-4.55 ± 1.04	5.5
B2-100	92	137.02 ± 69.77	0.21 ± 0.09	-8.02 ± 0.16	5.68
B3-100	88	225.48 ± 157.36	0.3 ± 0.2	-14.9 ± 2.9	5.97

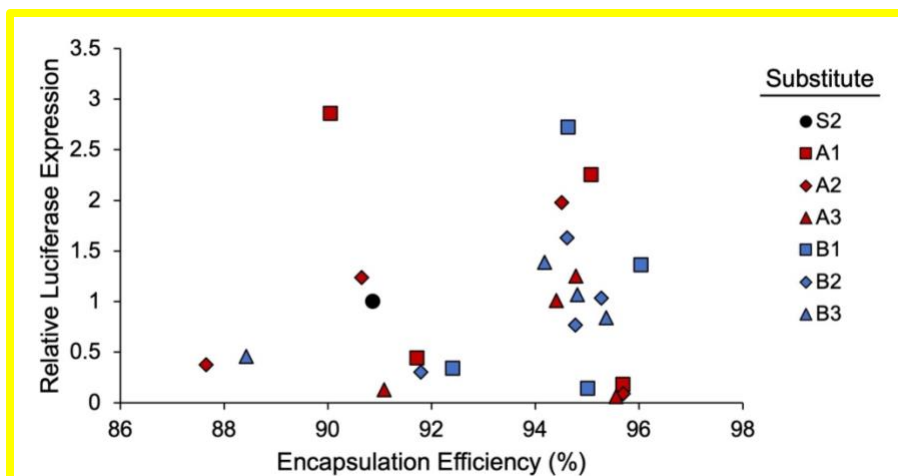
Supplemental Table 1. LNP library characterization data. Encapsulation efficiency, z-average size/diameter, PDI, zeta potential, and pKa for all LNPs in the library.



Supplemental Figure 1. Chemical structure of the C14-4 ionizable lipid.



Supplemental Figure 2. Luciferase expression in activated and unactivated primary T cells treated with luciferase-encoding mRNA LNPs with varying cholesterol substitutions. Primary human T cells were treated with various particles from the library (S2, A1-100, A2-100, A2-25, A3-25, B1-25, B2-25, B3-25) at a dose of 300 ng mRNA / 60,000 cells for 24 h. n = 1.



Supplemental Figure 3. Relationship between relative luciferase expression in Jurkats (immortalized T cells) and mRNA encapsulation efficiency of hydroxycholesterol-substituted LNPs and a control LNP. Replicate-averaged data from the in vitro screen described in the main text is plotted in this figure as relative luciferase expression versus mRNA encapsulation efficiency (%) to demonstrate no significant relationship between the two variables or hydroxycholesterol substitute. No correlation was found between the two variables in a t-test for linear regression ($p = 0.689 \gg 0.05$).